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Fraunhofer Gesellschaft...

SW-26022January 16, 2006Claims

1. A *C. albicans* cell containing a vector in which a nucleic acid molecule which encodes a cell wall protein necessary for the hyphae development of a pathogenic fungal organism is arranged in antisense orientation to at least one regulation elements and is selected from the group consisting of:

- a) a nucleic acid molecule having one of the nucleotide sequences shown in SEQ ID No. 1, SEQ ID No. 3 or SEQ ID No. 5,
- b) a nucleic acid molecule having a nucleotide sequence which encodes a protein having one of the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 4 or SEQ ID No. 6,
- c) a nucleic acid molecule having a nucleotide sequence which shows a homology of at least 80% to a

nucleotide sequence of one of the nucleic acid molecules of a) or b) over its entire length, and

d) a nucleic acid molecule having a nucleotide sequence which is complementary to a nucleotide sequence of one of the nucleic acid molecules of a) to c), and

e) a fragment of a nucleic acid molecule defined in a) to d) which can inhibit the expression of a cell wall protein of a pathogenic fungal organism in antisense orientation to a promoter in a host cell and comprises at least 10 nucleotides.

2. A method for the production of a cell wall protein necessary for the hyphae development of a pathogenic fungal organism, comprising the culturing of a host cell in a suitable culture medium under conditions which allow expression of the cell wall protein, and the obtainment of the expressed cell wall protein from the cell or from the medium, wherein the host cell contains at least one vector in which the nucleic acid molecule defined in claim 1 is arranged in antisense orientation to at least one regulation element.

3. An antibody which specifically recognizes a protein and binds thereto, wherein the protein contains the amino

acid sequence selected from SEQ ID No. 2, SEQ ID No. 4 and SEQ ID No. 6.

4. The antibody as claimed in claim 3, the antibody being a monoclonal or a polyclonal antibody.

5. A method for the characterization and/or for the detection of the hyphae stage of *Candida* cells or cells of pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species, comprising the incubation of the cells or cell fractions thereof with an agent for the identification of a cell wall protein which contains the amino acid sequence selected from SEQ ID No. 2, SEQ ID No. 4 and SEQ ID No. 6, the detection of the protein or of a fragment thereof indicating the presence of the virulent hyphae stage of the cells.

6. The method as claimed in claim 5, the *Candida* cells to be characterized being cells of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. glabrata*, *C. dubliniensis* or *C. lusitaniae*.

7. The method as claimed in claim 5 or 6, the cells to be characterized being present in a biological sample.

8. The method as claimed in one of claims 5 to 7, the cells to be characterized being cells isolated from a biological sample and enriched intact cells.

9. The method as claimed in one of claims 5 to 8, isolated cell fractions being employed for the characterization which are obtainable by cell disruption and fractionation of *Candida* cells or cells of species related to *Candida* and comprise at least one cell wall fraction.

9. The method as claimed in one of claims 5 to 9, the agent employed for the identification of the protein being an immunological agent.

10. The method as claimed in claim 9, the immunological agent being an antiserum directed against the protein, an antibody as claimed in claim 3 or 4 or a fragment thereof or a complex thereof.

11. The method as claimed in claim 10, the antibody having a label selected from the group consisting of a dye label, a radiolabel, a fluorescent label, a chemiluminescent label or an enzyme inducing a measurable reaction.

12. A method for the detection of a *Candida* infection and/or of an infection by pathogenic fungal organisms of a

Trichosporon species or of a *Blastoschizomyces* species in a biological sample obtained from a human or animal organism, the presence of the protein Rbr1p, Rbr2p and/or Rbr3p and/or of a fragment thereof in the biological sample and/or in the cell wall of *Candida* cells or cells of pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species optionally contained in the biological sample being detected, comprising

- a) the incubation of the biological sample with an agent for the identification of the cell wall protein which contains the amino acid sequence selected from SEQ ID No. 2, SEQ ID No. 4 and SEQ ID No. 6, and
- b) the detection of the interaction of the identification means with the protein.

13. The method as claimed in claim 12, the *Candida* cells being cells of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. glabrata*, *C. dubliniensis* or *C. lusitaniae*.

14. The method as claimed in claim 12 or 13, the biological sample being a skin or mucous membrane swab, an organ biopsy, a tissue biopsy, a body fluid, a body secretion, stool or a rinse from cavities or hollow organs.

15. The method as claimed in claim 14, the body fluid being sputum, urine, pleural effusion, spinal fluid, lymph or blood.

16. The method as claimed in claim 15, the blood being present as an unpurified blood sample, blood plasma or blood serum.

17. The method as claimed in claim 15 or 16, invasive candidiasis being detected by the detection of the protein in blood or in the cell wall of *Candida* cells contained in the blood.

18. The method as claimed in one of claims 12 to 17, the agent employed for the identification of the protein being an immunological agent.

19. The method as claimed in claim 18, the immunological agent being an antiserum directed against the protein, an antibody as claimed in claim 3 or 4 or a fragment thereof or a complex thereof.

20. The method as claimed in claim 18 or 19, the antibody having a label selected from the group consisting of a dye

label, a radiolabel, a fluorescent label, a chemiluminescent label or an enzyme inducing a measurable reaction.

21. A method for the discovery and identification of substances having therapeutic action against diseases which are caused by *Candida* species or pathogenic fungal *Trichosporon* or *Blastoschizomyces* species, a substance to be tested being brought into contact in a suitable medium with at least one agent and an interaction between the substance to be tested and the agent being detected, the agent being selected from the group consisting of:

a nucleic acid molecule which encodes a cell wall protein necessary for the hyphae development of a pathogenic fungal organism and is selected from the group consisting of:

- a) a nucleic acid molecule having one of the nucleotide sequences shown in SEQ ID No. 1, SEQ ID No. 3 or SEQ ID No. 5,
- b) a nucleic acid molecule having a nucleotide sequence which encodes a protein having one of the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 4 or SEQ ID No. 6,

c) a nucleic acid molecule having a nucleotide sequence which shows a homology of at least 80% to a nucleotide sequence of one of the nucleic acid molecules of a) or b) over its entire length,

d) a nucleic acid molecule having a nucleotide sequence which is complementary to a nucleotide sequence of one of the nucleic acid molecules of a) to c), and

e) a fragment of a nucleic acid molecule defined in a) to d) which can inhibit the expression of a cell wall protein of a pathogenic fungal organism in antisense orientation to a promoter in a host cell and comprises at least 10 nucleotides,

a vector which contains a nucleic acid molecule,

a host cell which contains the vector,

a protein which contains an amino acid sequence selected from SEQ ID No. 2, SEQ ID No. 4 and SEQ ID No. 6, and

an antibody which specifically recognizes the protein and binds thereto.

22. A diagnostic composition comprising an agent characterized in claim 21.

23. A pharmaceutical composition comprising an agent characterized in claim 21.

24. The pharmaceutical composition as claimed in claim 23, it being a vaccine which contains a protein characterized in claim 21 and which is suitable for the active immunization of a human or animal body against a *Candida* infection.

25. The pharmaceutical composition as claimed in claim 23, it being a vaccine which contains an antibody characterized in claim 21 and which is suitable for the passive immunization of a human or animal body against a *Candida* infection.

26. The pharmaceutical composition as claimed in claim 24 or 25, the vaccine being present as a lyophilizate.

27. The pharmaceutical composition as claimed in claim 24 or 25, the vaccine being present as an aqueous colloidal solution or suspension.

28. The pharmaceutical composition as claimed in one of claims 24 to 27, additionally containing at least one adjuvant.

29. A kit for the in vitro identification of the cell wall protein Rbr1p, Rbr2p and/or Rbr3p of *Candida* species or of a pathogenic organism of a *Trichosporon* species or of a *Blastoschizomyces* species and/or for the in vitro detection of the virulence of the cells, comprising at least one container having an antibody as claimed in claim 3 or 4.

30. The kit as claimed in claim 29, comprising a second container having the isolated and purified protein having one of the amino acid sequence shown in SEQ ID No. 2, SEQ ID No. 4 or SEQ ID No. 6.

31. The use of an agent characterized in claim 21 for the diagnosis of diseases of a human or animal organism which are caused by *Candida* species or pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species.

32. The use of an agent characterized in claim 21 for the production of a diagnostic composition for the diagnosis of diseases of a human or animal organism which are caused by *Candida* species or pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species.

33. The use of an agent characterized in claim 21 as an active compound for the treatment and/or prevention of

diseases of a human or animal organism which are caused by *Candida* species or pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species.

34. The use of an agent characterized in claim 21 as an active compound for the production of a pharmaceutical composition for the treatment and/or prevention of diseases of a human or animal organism which are caused by *Candida* species or pathogenic fungal organisms of a *Trichosporon* species or of a *Blastoschizomyces* species.

35. The use of an agent characterized in claim 21 for the identification and/or for the detection of substances which inhibit the expression or activity of the Rbr1p protein in a pathogenic fungal organism and are suitable as an active compound for the production of a pharmaceutical composition for the control of complaints caused by *Candida* species.

36. The use of a nucleic acid molecule having one of the nucleotide sequences shown in SEQ ID No. 1, SEQ ID No. 3 or SEQ ID No. 5, of a nucleic acid molecule having a nucleotide sequence which encodes a protein having one of the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 4 or SEQ ID No. 6, or of a fragment thereof for the isolation of a homologous nucleic acid which encodes the Rbr1p protein, the Rbr2p protein or Rbr3p protein of *C. tropicalis*, *C. krusei*, *C.*

parapsilosis, *C. guilliermondii*, *C. glabrata*, *C. dubliniensis*, *C. lusitaniae*, of a *Trichosporon* species, of a *Blastoschizomyces* species or of another fungal pathogenic organism.

37. The use of an antibody as claimed in claim 3 or 4 for the characterization and/or for the detection of the virulent hyphae stage of *Candida* cells.